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(57) Abstract

The present invention pertains to the prevention or lessening of disease in cats caused by Feline Immunodeficiency Virus (FIV). Prevention or lessening of disease is understood to mean the amelioration of any symptoms, including immune system disruptions, that result from FIV infection. The invention provides for a plasmid which encodes the FIV genome where said genome has had a portion of the *gag* gene, specifically the p10 (nucleocapsid) coding region, or a portion thereof, deleted. This deletion prevents the production of functional or whole p10 protein which, in turn, prevents the packaging of RNA into virions produced from transfection of this plasmid into an appropriate host cell, resulting in virions which do not contain RNA. Such virions will be described as "empty" virions. The invention also encompasses host cells transformed with the plasmid which produce the empty virions, and the empty virions themselves. In another embodiment, the invention encompasses vaccines that comprise one or more empty virions described above, with a pharmaceutically acceptable carrier or diluent and a pharmaceutically acceptable adjuvant. In yet another aspect, the invention provides methods for preventing or lessening disease caused by FIV, which is carried out by administering to a feline in need of such treatment the vaccines described above.

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GENETICALLY ALTERED FELINE IMMUNODEFICIENCY VIRUSES AND THEIR USE AS AN EFFECTIVE VACCINE AGAINST FELINE IMMUNODEFICIENCY VIRUS INFECTION

FIELD OF THE INVENTION

The present invention pertains to the prophylaxis and treatment of disease caused by feline immunodeficiency virus (FIV), using genetically altered FIV virions. Specifically, a portion of the p10 gene, which encodes a protein responsible for packaging of the RNA into the virion, has been deleted. The resulting virions are produced in appropriate host cell lines and used to make vaccines comprising whole killed virions which do not comprise viral RNA.

BACKGROUND OF THE INVENTION

Feline immunodeficiency virus (FIV) infection is a significant health problem for domestic cats around the world. As in its human counterpart, infection with FIV causes a progressive disruption in immune function. In the acute phase of infection, the virus causes transient illness associated with symptoms such as lymphadenopathy, pyrexia, and neutropenia. Subsequently, an infected animal enters an asymptomatic phase of 1-2 years before clinical manifestations of immune deficiency become apparent, after which the mean survival time is usually less than one year.

FIV is a typical retrovirus that contains a single-stranded polyadenylated RNA genome, internal structural proteins derived from the *gag* gene product, and a lipid envelope containing membrane proteins derived from the *env* gene product (Bendinelli et al., *Clin.Microbiol.Rev.* 8:87, 1995). The *gag* gene is translated into a primary product of

about 50 kDa that is subsequently cleaved by a viral protease into the matrix (p15), capsid (p25), and nucleocapsid (p10) proteins. The start and the end for each cleavage product of the GAG polyprotein are indicated in Figure 2 underneath the open reading frame. The *env* gene yields a primary translation product of 75-80 kDa (unglycosylated molecular weight); in infected cells, the precursor has an apparent molecular weight of 145-150 kDa due to N-linked glycosylation. The *env* precursor is cleaved in the Golgi apparatus into the SU and TM proteins (also designated gp95 and gp40, respectively).

As discussed above, the *gag* gene of the feline immunodeficiency virus (FIV) is initially translated as a precursor polyprotein which is cleaved to yield the functionally mature matrix protein, capsid protein and nucleocapsid protein making up the core of virus (Elder et al., J. Virol. 67: 1869-76, 1993). The *pol* gene overlaps the gag gene by 112 nucleotides, and is in a -1 reading frame with respect to that of the *gag* gene. Thus, the gene is translated as a Gag-Pol fusion protein produced by ribosome frameshifting. The overlapping region contains frameshift signals, GGGAAAC and GGAGAAAC, located at the 3' end of the *gag* gene (Morikawa et al., Virol. 186: 389-97, 1992).

The nucleocapsid protein, or p10, is a small basic protein, which is associated with the genomic RNA and may be required for viral RNA packaging (Egberink et al. J. Gen. Virol. 71: 739-743, 1990; Steinman et al., J. Gen. Virol. 71: 701-06, 1990). The p10 protein contains two cysteine arrays each consisting of 14 amino acid residues with the sequence $C\text{-}X_2\text{-}C\text{-}X_4\text{-}H\text{-}X_4\text{-}C$ (where X represents any amino acid and the subscript is the number of residues). Genetic studies with other retroviruses have shown that these two cysteine arrays are essential for viral RNA packaging (Rein et al., J. Virol. 68: 6124-29, 1994; Meric et al., J. Virol. 62: 3328-33; Gorelick et al., Proc. Natl. Acad. Sci. USA 85:8420-24, 1988). Therefore, deletion of these two cysteine arrays should, in theory, generate FIV virus particles which contains all viral proteins, but no viral genomic RNA. These FIV viral particles should be non-infectious and could be used to effect efficacious immune protection in vaccinated cats.

Most vaccines against FIV have failed to induce protective immunity. Ineffective vaccines have involved inactivated whole virus, fixed infected cells, recombinant CA and SU proteins, and a synthetic peptide corresponding to the V3 region

of SU. In some cases, the vaccine actually enhanced infection after challenge. In one system, vaccination with paraformaldehyde-fixed virus or infected cells resulted in protective immunity (Yamamoto et al., J. Virol. 67:601, 1993), but application of this approach by others was unsuccessful (Hosie et al., in Abstracts of the International Symposium on Feline Retrovirus Research, 1993, page 50).

Thus, there is a need in the art for an effective whole killed virion vaccine against FIV.

SUMMARY OF THE INVENTION

The present invention pertains to the prevention or lessening of disease in cats caused by Feline Immunodeficiency Virus (FIV). Prevention or lessening of disease is understood to mean the amelioration of any symptoms, including immune system disruptions, that result from FIV infection.

The invention provides for a plasmid which encodes the FIV genome where said genome has had a portion of the *gag* gene, specifically the p10 (nucleocapsid) coding region, or a portion thereof, deleted. This deletion prevents the production of functional or whole p10 protein, which in turn, prevents the packaging of RNA into virions produced from transfection of this plasmid into an appropriate host cell, resulting in virions which do not contain RNA. Such virions will be described as "empty" virions. The invention also encompasses host cells transformed with the plasmid which produce the empty virions, and the empty virions themselves.

In another embodiment, the invention encompasses vaccines that comprise one or more empty virions described above, with a pharmaceutically acceptable carrier or diluent and a pharmaceutically acceptable adjuvant.

In yet another aspect, the invention provides methods for preventing or lessening disease caused by FIV, which is carried out by administering to a feline in need of such treatment the vaccines described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphic illustration of the cloning strategy for creating FIV with deletion of p10.

Figure 2 shows the DNA sequence of the *gag* gene of FIV, [SEQ. I.D. NO. 5] with the delineations of the coding sequence for the various proteolytic products indicated. The double underlined DNA sequence is deleted in a preferred embodiment of the present invention.

Figure 3 shows the protein sequences for the translation products of the *gag* gene of FIV, including both the primary [SEQ. I.D. NO. 6] and secondary [SEQ. I.D. NO. 7] open reading frames. The double underlined amino acids are not encoded by a preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications, and references cited herein are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will control.

The vaccine of the present invention may be prepared by creating a recombinant FIV carrying a deletion of the p10 gene, or a portion thereof, encoding a portion of the *gag* protein of Feline Immunodeficiency Virus (FIV). The cloning scheme employed to produce the deleted virus eliminates 39 codons which include the two cysteine arrays within the p10 gene without disrupting either the *gag* gene open reading frame or the *gag-pol* frameshifting as occurs in the wild type virus-infected cells. The two cysteine arrays are highlighted in Figure 2, where cysteine array 1 encompasses nucleotides 1129 to 1170 and cysteine array 2 encompasses nucleotides 1186 to 1227. The thirty nine codons and amino acids which are deleted are double underlined in Figures 2 and 3. The deletion does not disrupt the original p10 open reading frame. The deletion also does not alter the gag-pol frameshift start site and frameshift signal. Therefore, in theory, the frequency of *gag-pol* frameshifting at nucleotide 1242 should not be affected by the deletion of the 39 codons preceding the *gag-pol* frameshift start site. Figure 2 indicates the *gag-pol* frameshift start site by single underlining. Figure 2 indicates the 5' end of the POL polyprotein underneath the p10 open reading frame, while Figure 3 lists the amino acid sequence of p10 and the frameshifted POL protein.

The process for constructing the p10 deletion vaccine is outlined as follows. A plasmid construct is made which deletes a portion of the p10 encoding gene sequences

using PCR-mediated mutagenesis. The construct is designed to not delete any of the 112 nucleotides (1243 to 1353) which overlap the *gag* and *pol* genes and to not eliminate the frameshift signal which is necessary for *pol* transcription. Once constructed, the plasmid is transfected into an appropriate host cell, such as mammalian cells, and the transformed cells are screened for non-infectious virus production. Cells which prove to produce non-infectious (presumably empty) virions are used to produce high levels of virus particles, which are isolated from the cell culture medium.

Although this particular construct and method are effective in producing empty virions, i.e., those which do not contain RNA, one of ordinary skill in the art would recognize alternative well-known methods of achieving the same goal. For example, the deletion need not eliminate the whole p10 encoding sequence, only enough sequence for the function of the protein to be eliminated. One representative example of this approach would be deletion of only one of the two cysteine arrays. Further, fragments of sequence need not be deleted. Any genetic alteration, i.e., site-directed mutagenesis of cysteines within the array, using methods well known in the art can be employed to construct a FIV genome which encodes empty virions. Thus, well-known variants of the genetic alterations presently employed which result in genomes which encode empty virions are contemplated to be within the scope of the present invention.

The isolated virus may be stored after concentration at 4°C or frozen (-50°C or colder) or lyophilized until the time of use. Compounds such as NZ-amine, dextrose, gelatin or others designed to stabilize the virus during freezing and lyophilization may be added. The virus may be concentrated using commercially available equipment. To produce the vaccine, isolated particles can be chemically treated to ensure lack of infectivity, that is, inactivated and mixed with an adjuvant(s).

Typically, the concentration of virus in the vaccine formulation will be a minimum of $10^{6.0}$ virus particles per dose, but will typically be in the range of $10^{6.0}$ to $10^{8.0}$ virus particles per dose. At the time of vaccination, the virus is thawed (if frozen) or reconstituted (if lyophilized) with a physiologically-acceptable carrier such as deionized water, saline, phosphate buffered saline, or the like. An additional optional component of the present vaccine is a pharmaceutically acceptable adjuvant. Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block

copolymers such as Pluronic® (L121) Saponin; detergents such as Tween -80; Quil® A, mineral oils such as Drakeol or Marcol®, vegetable oils such as peanut oil; Corynebacterium-derived adjuvants such as corynebacterium parvum; Propionibacterium-derived adjuvants such as Propionibacterium acne; Mycobacterium bovis (Bacillus Calmette and Guerinn, or BCG); interleukins such as interleukin 2 and interleukin-12; monokines such as interleukin 1; tumor necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminum hydroxide or Quil®-A aluminum hydroxide; liposomes; iscom adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A; dextran sulfate; DEAE-Dextran or DEAE-Dextran with aluminum phosphate; carboxypolymethylene, such as Carbopol®; ethylene malelic anhydride (EMA); acrylic copolymer emulsions such as Neocryl® A640 (e.g. U.S. Patent 5,047,238); vaccinia or animal poxvirus proteins; subviral particle adjuvants such as orbivirus; cholera toxin; dimethyldiocledecylammonium bromide; or mixtures thereof.

Individual genetically altered virions may be mixed together for vaccination. Furthermore, the virus may be mixed with additional inactivated or attenuated viruses, bacteria, or fungi such as feline leukemia virus, feline panleukopenia virus, feline rhinotracheitis virus, feline calicivirus, feline infectious peritonitis virus, feline *Chlamydia psittaci*, *Microsporum canis*, or others. In addition, antigens from the above-cited organisms may be incorporated into combination vaccines. These antigens may be purified from natural sources or from recombinant expression systems, or may comprise individual subunits of the antigen or synthetic peptides derived therefrom.

The produced vaccine can be administered to cats by subcutaneous, intramuscular, oral, intradermal, or intranasal routes. The number of injections and their temporal spacing may be varied. One to three vaccinations administered at intervals of one to three weeks are usually effective.

The efficacy of the vaccines of the present invention is assessed by the following methods. At about one month after the final vaccination, vaccinates and controls are each challenged with 3 - 20 cat ID_{50} units, preferably 5 cat ID_{50} units of FIV, preferably the NCSU-1 isolate (ATCC accession number VR 2333). Whole blood is obtained from the

animals immediately before challenge, and at intervals after challenge, for measurement of a) viremia and b) relative amounts of CD4 and CD8 lymphocytes.

Viremia is measured by isolating mononuclear cells from the blood, and co-culturing the cells with mononuclear cells from uninfected animals. After 7 days of culture, the culture supernatants are tested for FIV by enzyme-linked immunoassay (See Example 3 below).

The ratio of CD4 to CD8 lymphocytes in the circulation of vaccinates and controls is taken as a measure of immune function. Typically, FIV infection causes an inversion of the normal CD4:CD8 ratio of about 1.5-4 to a pathological ratio of about 0.5-1. The titers of CD4 and CD8 lymphocytes are measured by flow cytometry using specific antibodies (see Example 3 below).

Another measure of immune function is to challenge vaccinates and controls with *Toxoplasma gondii* at 6 months - 12 months after the final vaccination. Normally, the severity of *T. gondii*- induced disease symptoms is considerably exacerbated in FIV-infected cats relative to uninfected cats. The severity of the *T. gondii* effect is determined by scoring ocular discharge, nasal discharge, dyspnea, and fever.

It will be understood that amelioration of any of the symptoms of FIV infection is a desirable clinical goal. This includes a lessening of the dosage of medication used to treat FIV-induced symptoms.

The following examples are intended to illustrate the present invention without limitation thereof.

Example 1: Preparation of p10 deleted FIV strain

A. Isolation of Parental DNA

Purified lambda DNA containing the full length proviral sequence for the NCSU-1 isolate is prepared with Wizard Lambda Preps DNA Purification System (Promega Corporation, Madison, WI) and is used as the parental DNA for constructing deletion mutants. DNA digestion, ligation and other molecular techniques are performed as described (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, 1989).

B. Preparation of FIV-Left Plasmid

Purified lambda DNA is digested with SalI to release the 11-kb insert DNA containing the full length FIV proviral sequence. The insert DNA is purified by the glass bead method using the GENECLEAN II kit from BIO 101, Inc. and digested with NcoI which cuts only once on the FIV genome, producing a 2.9 kb SalI-NcoI fragment, designated as fragment A, and a 8.1 kb NcoI-SalI fragment, designated as fragment B.

Fragment A is purified by glass bead method as above and subcloned into plasmid vector pGEM 5Zf(t) (Promega Corp., Madison, WI) to generate plasmid pFIV-left. The plasmid pFIV-left contains the left portion of the viral genome including the LTR, p15, p25 and p10 gene.

C. Deletion of p10 Sequence

Deletion of the two cysteine arrays within the p10 gene is facilitated by PCR-mediated mutagenesis using high-fidelity *Pwo* DNA polymerase according to the manufacturer's manual (Boehringer Mannheim, USA, Indianapolis, IN). The plasmid pFIV-left is used as the initial template for PCR reaction. SP6 primer and primer A are used to amplify 2.2-kb fragment C with sequence which ends at nucleotide 1124. The SP6 primer:

5'-TTAGGTGACACTATAGAATACTCAA-3' [SEQ. I.D. NO. 1]

anneals to the vector sequence upstream the SalI site. Primer A:

5'-GGTCCTGATCCTTTTGATTGCACTA-3' [SEQ. I.D. NO. 2]

anneals to the FIV sequence, nucleotides 1100 to 1124.

Primer B and T7 primer are used to amplify 0.6-kb fragment D which starts at nucleotide 1242. The primer:

5'-AAAGAATTCGGGAAACTGGAAGGCGG-3' [SEQ. I.D. NO. 3]

anneals within the gag p10 gene, nucleotides 1242 to 1267. The T7 primer:

5'-TAATACGACTCACTATAGGGCGAATTG-3' [SEQ. I.D. NO. 4]

anneals to the vector sequence downstream from the NcoI site.

The location for each GAG-specific primer is highlighted in Figure 2.

Fragment C and fragment D are purified as above, ligated and the ligation products are used as the template to amplify a 2.8-kb fragment using SP6 primer and T7 primer.

The 2.8-kb fragment generated is purified as above and digested with SalI and NcoI to generate fragment E. Fragment E is identical to fragment A except the sequence for the segment spanning the two cysteine arrays is deleted, i.e. the sequence spanning nucleotides 1125 to 1241 is removed (see Fig. 1).

D. Construction of FIV delta p10 Plasmid

Fragment E and fragment B generated are purified as above. Then fragment E and fragment B are combined and cloned into the SalI site of the gene targeting vector pMC1neo Poly A (Stratagene, LaJolla, CA; Thomas, K. R., and Capecchi, M. R., Cell 51: 503-21, 1987), generating plasmid pFIV delta p10. The plasmid pFIV delta p10 contains the entire FIV genome with internal deletion within the p10 gene in addition to the neomycin resistance gene present on the gene targeting vector.

E. Production of Virions

Stable transfectants are obtained by transfecting the plasmid pFIV delta p10 into Vero cells (ATCC CCL 81), Crandell feline kidney cells (ATCC CCL 94) or AH927 feline embryonic fibroblast cells (Overbaugh et al., Virol. 188: 558-569, 1992) and selection by G418 by using cationic liposome-mediated transfection with the LIPOFECtamine® reagent and G418 (Genticin) according to the manufacturer's instruction (Life Technologies, Inc., Gaithersburg, MD). Cultures of G418-resistant cells are tested for virus particle production by a) assaying the viral particle-associated reverse transcriptase activity; b) complementation plaque assay as described (Rein et al., J. Virol. 29: 494-500, 1979) to determine if the virus particles are able to initiate single cycle of infection; c) Western blotting using antiserum against the major core protein p25 (IDEXX, USA, Portland, ME) to examine the integrity of the viral proteins; and d) direct examination of viral particles by electron microscopy.

The virus particles released from the stably transfected cells are to be examined for a) absence of viral RNA and DNA by RT-PCR and DNA PCR and b) absence of infectivity by the standard validated infectivity assays.

Example 2: Preparation of Whole Killed Empty FIV Vaccines

Stably-transfected cells which produce non-infectious viral particles are grown on microcarriers in bioreactors or in roller bottles. Culture fluids are harvested at the time or multiple times when the viral particles reach high levels as determined by electron microscopy and/or the feline immunodeficiency virus antigen test kit (IDEXX, USA, Portland, ME). The viral particles are inactivated by treatment with formalin or with binary ethylenimine, according to standard protocols well known in the art. Following inactivation, the viral particles are concentrated 10 to 50 fold with the hollow fiber procedure using a cut-off at molecular weight of 10,000 to 100,000 daltons. For preparing the vaccines, the concentrated fluids containing viral particles are mixed with immunologenically stimulating adjuvant, for example, ethylene maleic anhydride (EMA) 31, neocryl, MVP emulsigen, mineral oil, or adjuvant A or combination of several immunologenically stimulating adjuvants. Adjuvant A is an adjuvant comprising a block copolymer, such as a polyoxypropylene-polyoxyethylene (POP-POE) block copolymer, preferably Pluronic® L121 (e.g. U.S. Patent 4,772,466), and an organic component, such as a metabolizable oil, e.g. an unsaturated turpin hydrocarbon, preferably squalane (2,6,10,15,19,23-hexamethyltetracosane) or squalene.

In this adjuvant mixture, the block copolymer, organic oil, and surfactant may be present in amounts ranging from about 10 to about 40 ml/L, about 20 to about 80 ml/L, and about 1.5 to about 6.5 ml/L, respectively. In a preferred embodiment of the stock adjuvant, the organic component is squalane present in an amount of about 40 mL/L, the surfactant is polyoxyethylenesorbitan monooleate (Tween®-80) present in an amount of about 3.2 ml/L, and the POP-POE block copolymer is Pluronic® L121 present in an amount of about 20 ml/L. Pluronic® L121 is a liquid copolymer at 15-40 C, where the polyoxypropylene (POP) component has a molecular weight of 3250 to 4000 and the polyoxyethylene (POE) component comprises about 10-20%, preferably 10%, of the total molecule.

Non-limiting examples of other suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers such as Pluronic® (L121) Saponin; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol® or Marcol®, vegetable oils such as peanut oil; Corynebacterium-derived adjuvants such as corynebacterium parvum; Propionibacterium-derived adjuvants such as Propionibacterium

acne; Mycobacterium bovis (Bacillus Calmette and Guerinn, or BCG); interleukins such as interleukin 2 and interleukin-12; monokines such as interleukin 1; tumor necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminum hydroxide or Quil®-A aluminum hydroxide; liposomes; iscom adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A; dextran sulfate; DEAE-Dextran or DEAE-Dextran with aluminum phosphate; carboxypolymethylene, such as Carbopol®; EMA; acrylic copolymer emulsions such as Neocryl® A640 (e.g. U.S. Patent 5,047,238); vaccinia or animal poxvirus proteins; subviral particle adjuvants such as orbivirus; cholera toxin; dimethyldiocledecylammonium bromide; or mixtures thereof. The composition may also include a non-ionic detergent or surfactant, preferably a polyoxyethylene sorbitan monooleate such as a Tween® detergent, most preferably Tween®-80, i.e. polyoxyethylene (20) sorbitan monooleate.

Typically, 1 ml dose contains at least 10^6 viral particles, as determined by electron microscopy or the feline immunodeficiency virus antigen test kit (IDEXX, USA, Portland, ME).

Example 3: Test of Efficacy of Whole Killed Empty FIV Vaccines

A. *Vaccination*

Cats of age 8 weeks or greater are injected subcutaneously or intramascularly with the vaccine prepared above. Each cat receives two injections of vaccine at a 2-4 week interval. Two to six weeks following vaccination, the vaccinated cats and non-vaccinated cats are challenged by inoculating with 5 cat ID_{50} of feline immunodeficiency virus (NCSU-1 isolate (ATCC VR 2333) and some other isolates). Antibody response to vaccination is measured by ELISA using a neutralizing peptide within the immunodominant region (V3) of the FIV envelope protein (Lombardi et al., J. Virol. 67:4742-49, 1993). Viral replication following challenging is monitored biweekly by a) determining the levels of FIV RNA or/+ proviral DNA with RT-PCR and DNA PCR; and/or b) by co-cultivation for presence of infectious virus particles.

1. Detection of Viremia

a. PCR Detection of FIV proviral DNA

Mononuclear cells were isolated from whole blood using Percoll™ (Pharmacia Biotech, Piscataway NJ) gradients. 5 x 10^5 cells were lysed and 1/10th of the lysate used in a polymerase chain reaction assay with oligonucleotide primers specific to the gag gene of FIV (TL Wasmoen et al. Vet. Immun. Immunopath. 35: 83-93, 1992) or the equivalent. FIV amplified DNA was detected by agarose gel electrophoresis and ethidium bromide staining or by enzyme linked oligonucleotide assays.

b. Tissue Culture Isolation of FIV

Culture isolate of FIV is performed as described previously (Wasmoen et al., Vet. Immuno. Immunopath. 35:83-93, 1992). Mononuclear cells are isolated from whole blood using Percoll™ (Pharmacia Biotech, Piscataway NJ) gradients. 5 x 10^5 cells from FIV-challenged cats were cultured with 1 x 10^6 mononuclear cells isolated from uninfected cats. Cultures are fed with RPMI media every 7 days and supernatant tested for the presence of FIV by an enzyme-linked immunosorbent assay (ELISA) that detects FIV p25 antigen (Petcheck ELISA, IDEXX, Portland, ME). Alternatively, plasma can be used as the source of infectious virus.

2. Lymphocyte Subsets

Leukocytes are isolated from whole blood using Histopaque™ (Sigma Chemical Company, St. Louis MO) and lymphocyte subsets quantitated by staining the cells with antibodies specific to CD4 (monoclonal antibody CAT30A), CD8 (monoclonal antibody FLSM 3.357), pan T lymphocytes (monoclonal antibody FLSM 1.572) or B lymphocytes (anti-cat IgG) followed by FACS analysis. These monoclonal antibodies are described elsewhere (M.B. Tompkins et al. Vet. Immunol. Immunopathol. 26:305-317, 1990) and the flow cytometry procedure is the same as previously described (R.V. English et al. J. Infect. Dis. 170:543-552, 1994). CD4:CD8 ratios are calculated.

B. *Toxoplasma gondii* Challenge

Eight to twelve weeks following challenge with FIV, the cats are inoculated with 10,000 to 50,000 tacheozoites of *Toxoplasma gondii*. Tacheozoites of the ME49 strain of *T. gondii* that were frozen in 10% glycerol or oocyts were inoculated intraperitoneally into Swiss mice (Charles Rivers Laboratories) and serially passed in mice according to published

procedures (Davidson et al., *Am. J. Pathol.* 143:1486, 1993). Tacheozoites harvested from peritoneal fluids of mice were enumerated using a hemacytometer. Cats were tranquilized using ketamine hydrochloride and inoculated with 50,000 fresh tacheozoites into the right common carotid artery that had been surgically isolated. Inoculation with *Toxoplasma* in this dosage generally causes mortality in up to 50% of cats which are FIV-infected and have not been vaccinated. Following *Toxoplasma* challenge, cats are monitored weekly for signs of clinical disease including ocular discharge, nasal discharge, dyspnea, fever, depression, and weight loss for 3 days prior to and up to 48 days following *T. gondii* inoculation.

Clinical signs follow *T. gondii* challenge were scored as follows:

Clinical Sign		Score
Fever	103.0 to 103.9°F	1 point per day
	104.0 to 104.9°F	2 points per day
	≥105.0°F	3 points per day
(Temperatures were not scored until ≥1°F above baseline.)		
Depression/Lethargy		1 point per day
Dehydration		2 points per day
Nasal Discharge		1 point per day
Ocular Discharge		1 point per day
Respiratory Distress: Tachypnea		2 points per day
Dyspnea		4 points per day

It is expected that the vaccine prepared as described above will significantly reduce the appearance of clinical signs and mortality due to *Toxoplasma* infection.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Wasmoen, Terri
Chu, Hsien-Jue
Chavez, Lloyd

(ii) TITLE OF INVENTION: Recombinant Raccoon Pox Viruses and Their Use as an Effective Vaccine Against Feline Immunodeficiency Virus Infection

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: American Home Products Corporation
(B) STREET: 5 Giralda Farms
(C) CITY: Madison
(D) STATE: New Jersey
(E) COUNTRY: U.S.A.
(F) ZIP: 07940-0894

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Matthews, Gale F.
(B) REGISTRATION NUMBER: 32,269
(C) REFERENCE/DOCKET NUMBER: AHP-95065 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-660-6329
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(C) TELEX: 125751

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: 14

(viii) POSITION IN GENOME:
(B) MAP POSITION: 6637-6659
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TATAGAAGCA CCCCAAGAAG AG 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: 14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATTCCCCCA AAGTTATATT TC 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: 14

(viii) POSITION IN GENOME:
(B) MAP POSITION: 8264-8285
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAGTTACAT TAGAGCATCA AG 22

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: PPR

(viii) POSITION IN GENOME:
(B) MAP POSITION: 9126-9145
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCTAGATCT TCAGGGTCCC AATACTC 27

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: 14

(viii) POSITION IN GENOME:
(B) MAP POSITION: 610-630
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAATTCTAGA GAGACTCTAC AGCAACATG 29

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(B) STRAIN: 14

(viii) POSITION IN GENOME:
(B) MAP POSITION: 2005-2026
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TAATAGATCT GGCCTCTTTT CTAATGATG 29

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 471-493
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TATGGAAAAG GCAAGAGAAG GAC 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 763-785
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCGAGATACC ATGCTCTACA CTG 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 857-880
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATGGAAAAG ATGGGATGAG ACTA 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 1513-1535
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTCACTTACC TTCATAGTAA ACC 23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Gly Asn Gly Gln Gly Arg Asp Trp Lys Met Ala Ile Lys Arg Cys
1 5 10 15

Ser Asn Ala Ala Val Gly Val Gly Gly Lys Ser Lys Lys Phe Gly Glu
20 25 30

Gly Asn Phe Arg Trp Ala Ile Arg Met Ala Asn Val Ser Thr Gly Arg
35 40 45

Glu Pro Gly Asp Ile Pro Glu Thr Leu Asp Gln Leu Arg Leu Val Ile
50 55 60

Cys Asp Leu Gln Glu Arg Arg Lys Lys Phe Gly Ser Cys Lys Glu Ile
65 70 75 80

Asp Lys Ala Ile Val Thr Leu Lys Val Phe Ala Ala Val Gly Leu Leu
85 90 95

Asn Met Thr Val Ser Ser Ala Ala Ala Ala Glu Asn Met Phe Thr Gln
100 105 110

Met Gly Leu Asp Thr Arg Pro Ser Met Lys Glu Ala Gly Gly Lys Glu
115 120 125

Glu Gly Pro Pro Gln Ala Phe Pro Ile Gln Thr Val Asn Gly Val Pro
130 135 140

Gln Tyr Val Ala Leu Asp Pro Lys Met Val Ser Ile Phe Met Glu Lys
145 150 155 160

Ala Arg Glu Gly Leu Gly Gly Glu Glu Val Gln Leu Trp Phe Thr Ala
165 170 175

Phe Ser Ala Asn Leu Thr Pro Thr Asp Met Ala Thr Leu Ile Met Ala
180 185 190

Ala Pro Gly Cys Ala Ala Asp Lys Glu Ile Leu Asp Glu Ser Leu Lys
195 200 205

Gln Leu Thr Ala Gly Tyr Asp Arg Thr His Pro Pro Asp Ala Pro Arg
210 215 220

Pro Leu Pro Tyr Phe Thr Ala Ala Glu Ile Met Gly Ile Gly Phe Thr
225 230 235 240

Gln Glu Gln Gln Ala Glu Ala Arg Phe Ala Pro Ala Arg Met Gln Cys
245 250 255

Arg Ala Trp Tyr Leu Glu Gly Leu Gly Lys Leu Gly Ala Ile Lys Ala
260 265 270

Lys Ser Pro Arg Ala Val Gln Leu Arg Gln Gly Ala Lys Glu Asp Tyr
275 280 285

Ser Ser Phe Ile Asp Arg Leu Phe Ala Gln Ile Asp Gln Glu Gln Asn
290 295 300

Thr Ala Glu Val Lys Leu Tyr Leu Lys Gln Ser Leu Ser Met Ala Asn
305 310 315 320

Ala Asn Ala Glu Cys Lys Lys Pro Met Thr His Leu Lys Pro Glu Ser
325 330 335

Thr Leu Glu Glu Lys Leu Arg Ala Cys Gln Glu Ile Gly Ser Pro Gly
340 345 350

Tyr Lys Met Gln Leu Leu Ala Glu Ala Leu Thr Lys Val Gln Val Val
355 360 365

Gln Ser Lys Gly Ser Gly Pro Val Cys Phe Asn Cys Lys Lys Pro Gly
370 375 380

His Leu Ala Arg Gln Cys Arg Glu Val Arg Lys Cys Asn Lys Cys Gly
385 390 395 400

Lys Pro Gly His Val Ala Ala Lys Cys Trp Gln Gly Asn Arg Lys Asn
405 410 415

Ser Gly Asn Trp Lys Ala Gly Arg Ala Ala Ala Pro Val Asn Gln Val
420 425 430

Gln Gln Ala Val Met Pro Ser Pro Pro Met Glu Glu Lys Leu Leu Asp
435 440 445

Leu

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 855 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Glu Gly Phe Ala Ala Asn Arg Gln Trp Ile Gly Glu Glu Ala
1 5 10 15

Glu Glu Leu Leu Asp Phe Asp Ile Ala Thr Gln Met Asn Glu Glu Gly
20 25 30

Pro Leu Asn Pro Gly Met Asn Pro Phe Arg Val Pro Gly Ile Thr Asp
35 40 45

Lys Glu Lys Gln Asp Tyr Cys Asn Ile Leu Gln Pro Lys Leu Gln Asp
50 55 60

Leu Arg Asn Glu Leu Gln Glu Val Lys Leu Glu Glu Gly Asn Ala Gly
65 70 75 80

Lys Phe Arg Arg Thr Arg Phe Leu Arg Tyr Ser Asp Glu Gln Val Leu
85 90 95

Ser Pro Val His Ala Phe Ile Gly Tyr Cys Ile Tyr Leu Gly Asn Arg
100 105 110

Asn Lys Leu Gly Ser Leu Arg His Asp Ile Asp Ile Glu Ala Pro Pro
115 120 125

Glu Glu Cys Tyr Asp Asn Arg Glu Lys Gly Thr Thr Asp Asn Ile Lys
130 135 140

Tyr Gly Arg Arg Cys Cys Leu Gly Thr Val Thr Leu Tyr Leu Ile Leu
145 150 155 160

Phe Ile Gly Leu Ile Ile Tyr Ser Gln Thr Ala Asp Ala Gln Val Val
165 170 175

Trp Arg Leu Pro Pro Leu Val Val Pro Val Glu Glu Ser Glu Ile Ile
180 185 190

Phe Trp Asp Cys Trp Ala Pro Glu Glu Pro Ala Cys Gln Asp Phe Leu
195 200 205

Gly Ala Met Ile His Leu Lys Ala Lys Thr Asn Ile Ser Ile Arg Glu
210 215 220

Gly Pro Thr Leu Gly Asn Trp Ala Arg Glu Ile Trp Ala Thr Leu Phe
225 230 235 240

Lys Lys Ala Thr Arg Gln Cys Arg Arg Gly Arg Ile Trp Lys Arg Trp
245 250 255

Asp Glu Thr Ile Thr Gly Pro Ser Gly Cys Ala Asn Asn Thr Cys Tyr
260 265 270

Asn Val Ser Ala Ile Val Pro Asp Tyr Gln Arg Tyr Leu Asp Arg Val
275 280 285

Asp Thr Trp Leu Gln Gly Lys Ile Asn Ile Ser Leu Cys Leu Thr Gly
290 295 300

Gly Lys Met Leu Tyr Asn Lys Val Thr Lys Gln Leu Ser Tyr Cys Thr
305 310 315 320

Asp Pro Leu Gln Ile Pro Leu Ile Asn Tyr Thr Phe Gly Pro Asn Gln
325 330 335

Thr Cys Met Trp Asn Thr Ser Gln Ile Gln Asp Pro Glu Ile Pro Gln
340 345 350

Cys Gly Trp Trp Asn His Met Ala Tyr Tyr Asn Ser Cys Lys Trp Glu
355 360 365

Glu Ala Lys Val Lys Phe His Cys Gln Arg Thr Gln Ser Gln Pro Gly
370 375 380

Ser Trp Arg Arg Ala Ile Ser Ser Trp Lys Gln Arg Asn Arg Trp Glu
385 390 395 400

Trp Arg Pro Asp Phe Glu Ser Glu Lys Val Lys Ile Ser Leu Gln Cys
405 410 415

Asn Ser Thr Lys Asn Leu Thr Phe Ala Met Arg Ser Ser Gly Asp Tyr
420 425 430

Gly Glu Val Thr Gly Ala Trp Ile Glu Phe Gly Cys His Arg Asn Lys
435 440 445

Ser Asn Leu His Thr Glu Ala Arg Phe Arg Ile Arg Cys Arg Trp Asn
450 455 460

Val Gly Ser Asp Thr Ser Leu Ile Asp Thr Cys Gly Asn Thr Pro Asn
465 470 475 480

Val Ser Gly Ala Asn Pro Val Asp Cys Thr Met Tyr Ser Asn Lys Met
485 490 495

Tyr Lys Phe Ser Leu Pro Asn Gly Phe Thr Met Lys Val Asp Asp Leu
500 505 510

Ile Met His Phe Asn Met Pro Lys Ala Val Glu Met Asn Asn Ile Ala
515 520 525

Gly Asn Trp Ser Cys Thr Ser Asp Leu Pro Ser Ser Trp Gly Tyr Met
530 535 540

Asn Cys Asn Cys Pro Asn Ser Ser Ser Ser Tyr Ser Gly Thr Lys Met
545 550 555 560

Ala Cys Pro Ser Asn Arg Gly Ile Leu Arg Asn Trp Tyr Asn Pro Val
565 570 575

Ala Gly Leu Arg Gln Ser Leu Glu Gln Tyr Gln Val Val Lys Gln Pro
580 585 590

Asp Tyr Leu Leu Val Pro Glu Glu Val Met Glu Tyr Lys Pro Arg Arg
595 600 605

Lys Arg Ala Ala Ile His Val Met Leu Ala Leu Ala Thr Val Leu Ser
610 615 620

Ile Ala Gly Ala Gly Thr Gly Ala Thr Ala Ile Gly Met Val Thr Gln
625 630 635 640

Tyr His Gln Val Leu Ala Thr His Gln Glu Ser Met Glu Lys Val Thr
645 650 655

Glu Ala Leu Glu Ile Asn Asn Leu Arg Leu Val Thr Leu Glu His Gln
660 665 670

Val Leu Val Ile Gly Leu Lys Val Glu Ala Met Glu Lys Phe Leu Tyr
675 680 685

Thr Ala Phe Ala Met Gln Glu Leu Gly Cys Asn Pro Asn Gln Phe Phe
690 695 700

Ser Lys Ile Pro Leu Glu Leu Trp Thr Arg Tyr Asn Met Thr Ile Asn
705 710 715 720

Gln Thr Ile Trp Asn His Gly Asn Ile Thr Leu Gly Glu Trp Tyr Asn
725 730 735

His Thr Lys Asp Leu Gln Pro Lys Phe Tyr Glu Ile Ile Met Asp Ile
740 745 750

Glu Pro Asn Asn Val Gln Gly Lys Thr Gly Ile Gln Gln Leu Pro Lys
755 760 765

Trp Glu Asp Trp Val Arg Trp Ile Gly Asn Ile Pro Gln Tyr Leu Lys
770 775 780

Gly Leu Leu Gly Gly Ile Leu Gly Ile Gly Leu Gly Val Leu Leu Leu
785 790 795 800

Ile Leu Cys Leu Pro Thr Leu Val Asp Cys Ile Arg Asn Cys Ile His
805 810 815

Lys Ile Leu Gly Tyr Thr Val Ile Ala Met Pro Glu Val Glu Gly Glu
820 825 830

Glu Ile Gln Pro Gln Met Glu Leu Arg Arg Asn Gly Ser Gln Phe Gly
835 840 845

Met Ser Glu Lys Glu Glu Glu
850 855

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 1-1353
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGGGAATG GACAGGGGCG AGATTGGAAA ATGGCCATTA AGAGATGTAG TAATGCTGCT 60

GTAGGAGTAG GGGGGAAGAG TAAAAAATTT GGGGAAGGGA ATTTCAGATG GGCCATTAGA 120

ATGGCTAATG TATCTACAGG ACGAGAACCT GGTGATATAC CAGAGACTTT AGATCAACTA 180

AGGTTGGTTA TTTGCGATTT ACAAGAAAGA AGAAAAAAAT TTGGATCTTG CAAAGAAATT 240

GATAAGGCAA TTGTTACATT AAAAGTCTTT GCGGCAGTAG GACTTTTAAA TATGACAGTG 300

TCTTCTGCTG CTGCAGCTGA AAATATGTTC ACTCAGATGG GATTAGACAC TAGACCATCT 360

ATGAAAGAAG CAGGAGGAAA AGAGGAAGGC CCTCCACAGG CATTTCCTAT TCAAACAGTA 420

AATGGAGTAC CACAATATGT AGCACTTGAC CCAAAAATGG TGTCCATTTT TATGGAAAAG 480

GCAAGAGAAG GATTAGGAGG TGAGGAAGTT CAGCTATGGT TCACTGCCTT CTCTGCAAAT 540

TTAACACCTA CTGACATGGC CACATTAATA ATGGCCGCAC CAGGGTGCGC TGCAGATAAA 600

GAAATATTGG ATGAAAGCTT AAAGCAACTT ACTGCAGGAT ATGATCGTAC ACATCCCCCT 660

GATGCTCCCA GACCATTACC CTATTTTACT GCAGCAGAAA TTATGGGTAT TGGATTTACT 720

CAAGAACAAC AAGCAGAAGC AAGATTTGCA CCAGCTAGGA TGCAGTGTAG AGCATGGTAT 780

CTCGAGGGAC TAGGAAAATT GGGCGCCATA AAAGCTAAGT CTCCTCGAGC TGTGCAGTTA 840

AGACAAGGAG CTAAGGAAGA TTATTCATCC TTTATTGACA GATTGTTTGC CCAAATAGAT 900

CAAGAACAAA ATACAGCTGA AGTTAAGTTA TATTTAAAAC AGTCATTAAG CATGGCTAAT 960

GCTAATGCAG AATGTAAAAA GCCAATGACC CACCTTAAGC CAGAAAGTAC CCTAGAAGAA 1020

AAGTTGAGAG CTTGTCAAGA AATAGGCTCA CCAGGATATA AAATGCAACT CTTGGCAGAA 1080

GCTCTTACAA AAGTTCAAGT AGTGCAATCA AAAGGATCAG GACCAGTGTG TTTTAATTGT 1140

AAAAAACCAG GACATCTAGC AAGACAATGT AGAGAAGTGA GAAAATGTAA TAAATGTGGA 1200

AAACCTGGTC ATGTAGCTGC CAAATGTTGG CAAGGAAATA GAAAGAATTC GGGAAACTGG 1260

AAGGCGGGGC GAGCTGCAGC CCCAGTGAAT CAAGTGCAGC AAGCAGTAAT GCCATCTGCA 1320

CCTCCAATGG AGGAGAAACT ATTGGATTTA TAA 1353

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Feline immunodeficiency virus
(C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:
(B) MAP POSITION: 1-3225
(C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGATCCAACA ATAATTATGG CAGAAGGATT TGCAGCCAAT AGACAATGGA TAGGACCAGA 60

AGAAGCTGAA GAGTTATTAG ATTTTGATAT AGCAACACAA ATGAATGAAG AAGGGCCACT 120

AAATCCAGGG ATGAACCCAT TTAGGGTACC TGGAATAACA GATAAAGAAA AGCAAGACTA 180

TTGTAACATA TTACAACCTA AGTTACAAGA TTTACGGAAT GAACTTCAAG AGGTAAAACT 240

AGAAGAAGGA AATGCAGGTA AGTTTAGAAG AACAAGATTT TTAAGGTATT CTGATGAACA 300

AGTATTGTCC CCGGTTCATG CGTTCATAGG ATATTGTATT TATTTAGGTA ATCGAAATAA 360

GTTAGGATCT TTAAGACATG ACATTGATAT TGAAGCACCC CCCGAAGAGT GTTATGATAA 420

TAGAGAGAAG GGTACAACTG ACAATATAAA ATATGGTAGA CGATGTTGCC TAGGAACGGT 480

GACTTTGTAC CTGATTTTAT TTATAGGATT AATAATATAT TCACAGACAG CCGACGCTCA 540

GGTAGTATGG AGACTTCCAC CATTAGTAGT CCCAGTAGAA GAATCAGAAA TAATTTTTTG 600

GGATTGTTGG GCACCAGAAG AACCCGCCTG TCAGGACTTT CTTGGGGCAA TGATACATCT 660

AAAAGCTAAG ACAAATATAA GTATACGAGA GGGACCTACC TTGGGGAATT GGGCTAGAGA 720

AATATGGGCA ACATTATTCA AAAAGGCTAC TAGACAATGT AGAAGAGGCA GAATATGGAA 780

AAGATGGGAT GAGACTATAA CAGGACCATC AGGATGTGCT AATAACACAT GTTATAATGT 840

TTCAGCAATA GTACCTGATT ATCAGCGTTA TTTAGATAGA GTAGATACTT GGTTACAAGG 900

GAAAATAAAT ATATCATTAT GTCTAACAGG AGGAAAAATG TTGTACAATA AAGTTACAAA 960

ACAATTAAGC TATTGTACAG ACCCATTACA AATCCCACTG ATCAATTATA CATTTGGACC 1020

TAATCAAACA TGTATGTGGA ATACTTCACA AATTCAGGAC CCTGAAATAC CACAATGTGG 1080

ATGGTGGAAT CACATGGCCT ATTATAACAG TTGTAAATGG GAAGAGGCAA AGGTAAAGTT 1140

TCATTGTCAA AGAACACAGA GTCAGCCTGG GTCATGGCGT AGAGCAATCT CGTCATGGAA 1200

ACAAAGAAAT AGATGGGAGT GGAGACCAGA TTTTGAGAGT GAAAAGGTGA AAATATCTCT 1260

ACAGTGCAAT AGCACGAAAA ACCTAACCTT TGCAATGAGA AGTTCAGGAG ATTATGGAGA 1320

AGTAACGGGA GCTTGGATAG AGTTTGGATG TCATAGAAAT AAATCAAACC TTCATACTGA 1380

AGCAAGGTTT AGAATTAGAT GTAGATGGAA TGTAGGGAGT GATACCTCGC TCATTGATAC 1440

ATGTGGAAAC ACTCCAAATG TTTCAGGTGC GAATCCTGTA GATTGTACCA TGTATTCAAA 1500

TAAAATGTAC AAGTTTTCTT TACCAAACGG GTTTACAATG AAGGTAGATG ACCTTATTAT 1560

GCATTTCAAT ATGCCAAAAG CTGTAGAAAT GAATAATATT GCTGGAAATT GGTCTTGTAC 1620

ATCTGACTTG CCATCGTCAT GGGGGTATAT GAATTGTAAT TGCCCAAATA GTAGTAGTAG 1680

TTATAGTGGT ACTAAAATGG CATGTCCTAG CAATCGAGGC ATCTTAAGGA ATTGGTATAA 1740

CCCAGTAGCA GGATTACGAC AATCCTTAGA ACAGTATCAA GTTGTAAAAC AACCAGATTA 1800

CTTACTGGTC CCAGAGGAAG TCATGGAATA TAAACCTAGA AGGAAAAGGG CAGCTATTCA 1860

TGTTATGTTG GCTCTTGCAA CAGTATTATC TATTGCCGGT GCAGGGACGG GGGCTACTGC 1920

TATAGGGATG GTAACACAAT ACCACCAAGT TCTGGCAACC CATCAAGAAT CTATGGAAAA 1980

GGTGACTGAA GCCTTAGAGA TAAACAACTT AAGGTTAGTT ACATTAGAGC ATCAAGTACT 2040

AGTAATAGGA TTAAAAGTAG AAGCTATGGA AAAATTTTTA TATACAGCTT TCGCTATGCA 2100

AGAATTAGGA TGTAATCCAA ATCAATTTTT CTCCAAAATC CCTCTTGAGT TGTGGACAAG 2160

GTATAATATG ACTATAAATC AAACAATATG GAATCATGGA AATATAACTT TGGGGGAATG 2220

GTATAACCAC ACCAAAGATT TACAACCAAA GTTTTATGAA ATAATAATGG ACATAGAACC 2280

AAATAATGTA CAAGGGAAAA CAGGGATACA ACAATTACCC AAGTGGGAAG ATTGGGTAAG 2340

ATGGATAGGA AATATTCCAC AATATTTAAA GGGACTATTG GGAGGTATCT TGGGAATAGG 2400

ATTAGGAGTG TTATTATTGA TTTTATGTTT ACCTACATTG GTTGATTGTA TAAGAAATTG 2460

TATCCACAAG ATACTAGGAT ACACAGTAAT TGCAATGCCT GAAGTAGAAG GAGAAGAAAT 2520

ACAACCACAA ATGGAATTGA GGAGAAATGG TAGCCAATTT GGCATGTCTG AAAAAGAGGA 2580

GGAATGATGA AGTATCTCAG ACTTATTTTA TAAGGGAGAT ACTGTGCTAA GTTCTTCCCT 2640

TTGAGGAAGG TATGTCATAT GAATCCATTT CGAACCAAAT CAAACTAATA AAGTATGTAT 2700

TGTAAGGTAA AAGGAAAAGA CAAAGAAGAA GAAGAAAGAA GAAAGCTTTC AAGAGGATGA 2760

TGACAGAGTT AGAAGATCGC TTCAGGAAGC TATTTGGCAC GACTTCTACA ACGGGAGACA 2820

GCACAGTAGA TTCTGAAGAT GAACCTCCTA AAAAAGAAAA AAGGGTGGAC TGGGATGAGT 2880

ATTGGAACCC TGAAGAAATA GAAAGAATGC TTATGGACTA GGGACTGTTT ACGAACAAAT 2940

GATAAAAGGA AATAGCTAAG CATGACTCAT AGTTAAAGCG CTAGCAGCTG CTTAACCGCA 3000

AAACCACATC CTATGTAAAG CTTGCTAATG ACGTATAAGT TGTTCCATTG TAAGAGTATA 3060

TAACCAGTGC TTTGTGAAAC TTCGAGGAGT CTCTCCGTTG AGGACTTTCG AGTTCTCCCT 3120

TGAGGCTCCC ACAGATACAA TAAATATTTG AGATTGAACC CTGTCAAGTA TCTGTGTAAT 3180

CTTTTTTACC TGTGAGGTCT CGGAATCCGG GCCGAGAACT TCGCA 3225

What is claimed is:

1. A plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.

2. The plasmid of claim 1 wherein said deletion of nucleotides encoding the FIV p10 protein are as set out in Figure 2.

3. The plasmid of claim 2 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.

4. A vaccine comprising virions of Feline Immunodeficiency Virus (FIV) which do not comprise whole p10 nucleocapsid protein.

5. The vaccine of claim 4 further comprising a pharmaceutically acceptable adjuvant.

6. The vaccine of claim 4 wherein said virions are produced from transfection of appropriate host cells by a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof and a pharmaceutically acceptable carrier or diluent.

7. The vaccine of claim 4 wherein said deletion of nucleotides encoding the FIV p10 protein is as set out in Figure 2.

8. The vaccine of claim 4 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.

9. The vaccine of claim 4 further comprising immunogens derived from viruses selected from the group consisting of feline leukemia virus, feline panleucopenia virus, feline rhinotracheitis virus, feline calicivirus, feline infectious peritoneal virus, feline herpesvirus, feline enteric coronavirus, or mixtures thereof.

10. The vaccine of claim 4 further comprising inactivated or attenuated feline *Chlamydia psittaci, Microsporum canis*, or mixtures thereof.

11. A FIV virion which does not comprise whole p10 nucleocapsid protein.

12. The FIV virion of claim 10 which was produced by transfection of appropriate host cells with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.

13. Host cells which are transfected with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof, such that said cells produce FIV virions which do not comprise whole p10 nucleocapsid protein.

14. Transfected host cells of claim 12 which are selected from the group consisting of Vero cells (ATCC CCL 81), Crandell feline kidney cells (ATCC CCL 94), and AH927 feline embryonic fibroblast cells.

15. A method for preventing or lessening disease caused by Feline Immunodeficiency Virus (FIV), comprising administering to a feline in need of such treatment vaccine comprising FIV virions which do not comprise whole p10 nucleocapsid protein.

16. The method of claim 14 wherein said virion was produced by transfection of appropriate host cells with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.

17. The method of claim 14 wherein said deletion of nucleotides encoding the FIV p10 protein is as set out in Figure 2.

18. The method of claim 15 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.

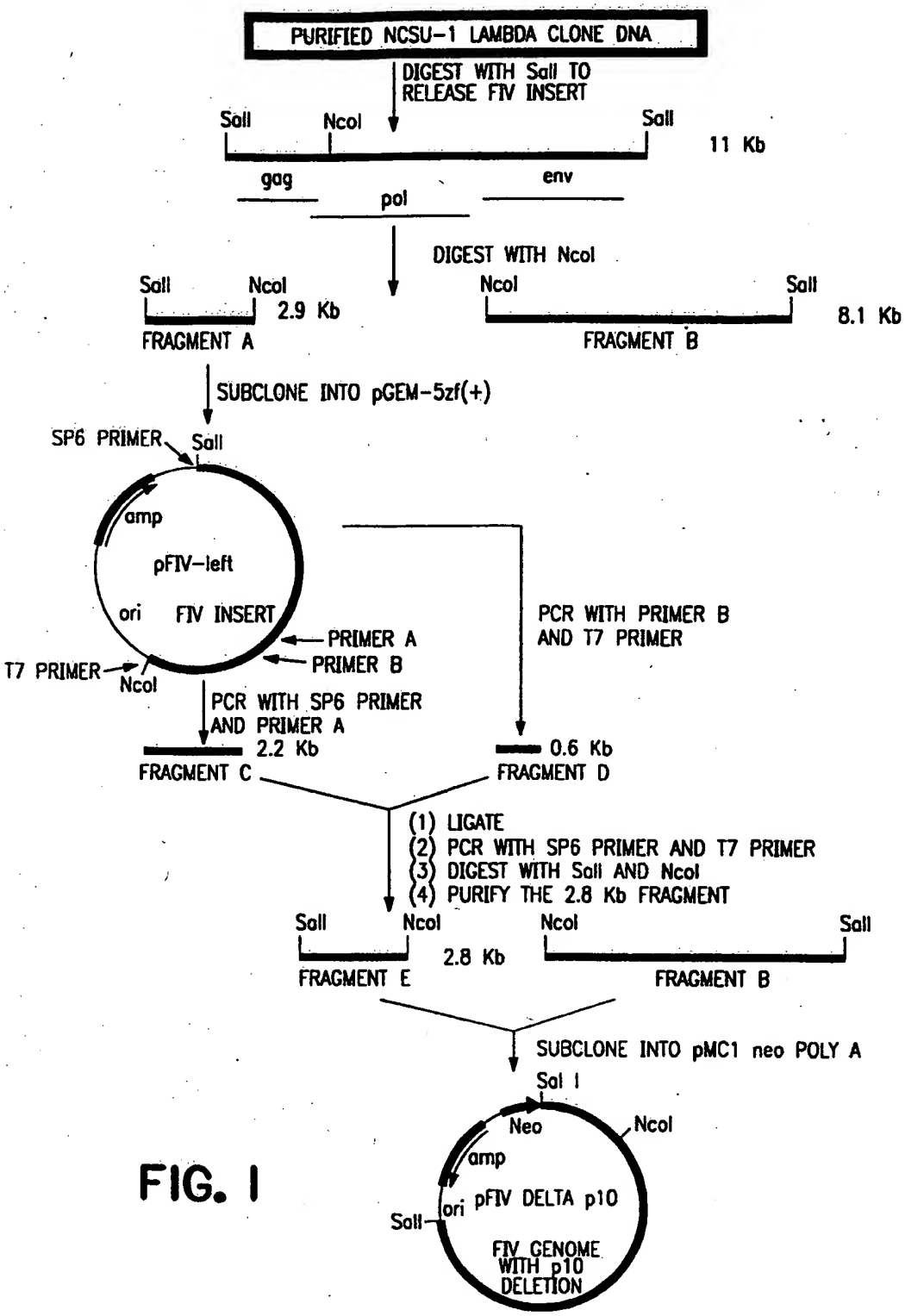
PURIFIED NCSU-1 LAMBDA CLONE DNA
DIGEST WITH SalI TO RELEASE FIV INSERT
SalI
NcoI
SalI
11 Kb
gag
pol
env
DIGEST WITH NcoI
SalI
NcoI
2.9 Kb
FRAGMENT A
NcoI
SalI
8.1 Kb
FRAGMENT B
SUBCLONE INTO pGEM-5zf(+)
SP6 PRIMER
SalI
amp
pFIV-left
ori
FIV INSERT
PRIMER A
PRIMER B
T7 PRIMER
NcoI
PCR WITH PRIMER B AND T7 PRIMER
PCR WITH SP6 PRIMER AND PRIMER A
2.2 Kb
FRAGMENT C
0.6 Kb
FRAGMENT D
(1) LIGATE
(2) PCR WITH SP6 PRIMER AND T7 PRIMER
(3) DIGEST WITH SalI AND NcoI
(4) PURIFY THE 2.8 Kb FRAGMENT
SalI
NcoI
2.8 Kb
FRAGMENT E
NcoI
SalI
FRAGMENT B
SUBCLONE INTO pMC1 neo POLY A
Sal I
Neo
NcoI
amp
ori
pFIV DELTA p10
SalI
FIV GENOME WITH p10 DELETION

FIG. 1

Sequence Range: 1 to 1353

```
      10         20         30         40         50         60
       *          *          *          *          *          *
ATGGGGAATG GACAGGGGCG AGATTGGAAA ATGGCCATTA AGAGATGTAG TAATGCTGCT
TACCCCTTAC CTGTCCCCGC TCTAACCTTT TACCGGTAAT TCTCTACATC ATTACGACGA
-----> p15 Matrix protein

      70         80         90        100        110        120
       *          *          *          *          *          *
GTAGGAGTAG GGGGGAAGAG TAAAAAATTT GGGGAAGGGA ATTTCAGATG GGCCATTAGA
CATCCTCATC CCCCCTTCTC ATTTTTTAAA CCCCTTCCCT TAAAGTCTAC CCGGTAATCT

     130        140        150        160        170        180
       *          *          *          *          *          *
ATGGCTAATG TATCTACAGG ACGAGAACCT GGTGATATAC CAGAGACTTT AGATCAACTA
TACCGATTAC ATAGATGTCC TGCTCTTGGA CCACTATATG GTCTCTGAAA TCTAGTTGAT

     190        200        210        220        230        240
       *          *          *          *          *          *
AGGTTGGTTA TTTGCGATTT ACAAGAAAGA AGAAAAAAAT TTGGATCTTG CAAAGAAATT
TCCAACCAAT AAACGCTAAA TGTTCTTTCT TCTTTTTTTA AACCTAGAAC GTTTCTTTAA

     250        260        270        280        290        300
       *          *          *          *          *          *
GATAAGGCAA TTGTTACATT AAAAGTCTTT GCGGCAGTAG GACTTTTAAA TATGACAGTG
CTATTCCGTT AACAATGTAA TTTTCAGAAA CGCCGTCATC CTGAAAATTT ATACTGTCAC

     310        320        330        340        350        360
       *          *          *          *          *          *
TCTTCTGCTG CTGCAGCTGA AAATATGTTC ACTCAGATGG GATTAGACAC TAGACCATCT
AGAAGACGAC GACGTCGACT TTTATACAAG TGAGTCTACC CTAATCTGTG ATCTGGTAGA

     370        380        390        400        410        420
       *          *          *          *          *          *
ATGAAAGAAG CAGGAGGAAA AGAGGAAGGC CCTCCACAGG CATTTCCTAT TCAAACAGTA
TACTTTCTTC GTCCTCCTTT TCTCCTTCCG GGAGGTGTCC GTAAAGGATA AGTTTGTCAT
                                p15 <-------  ------> p25
                                              Capsid protein

     430        440        450        460        470        480
       *          *          *          *          *          *
AATGGAGTAC CACAATATGT AGCACTTGAC CCAAAAATGG TGTCCATTTT TATGGAAAAG
TTACCTCATG GTGTTATACA TCGTGAACTG GGTTTTTACC ACAGGTAAAA ATACCTTTTC

     490        500        510        520        530        540
       *          *          *          *          *          *
GCAAGAGAAG GATTAGGAGG TGAGGAAGTT CAGCTATGGT TCACTGCCTT CTCTGCAAAT
CGTTCTCTTC CTAATCCTCC ACTCCTTCAA GTCGATACCA AGTGACGGAA GAGACGTTTA

     550        560        570        580        590        600
       *          *          *          *          *          *
TTAACACCTA CTGACATGGC CACATTAATA ATGGCCGCAC CAGGGTGCGC TGCAGATAAA
AATTGTGGAT GACTGTACCG GTGTAATTAT TACCGGCGTG GTCCCACGCG ACGTCTATTT

     610        620        630        640        650        660
       *          *          *          *          *          *
GAAATATTGG ATGAAAGCTT AAAGCAACTT ACTGCAGGAT ATGATCGTAC ACATCCCCCT
CTTTATAACC TACTTTCGAA TTTCGTTGAA TGACGTCCTA TACTAGCATG TGTAGGGGGA

     670        680        690        700        710        720
       *          *          *          *          *          *
GATGCTCCCA GACCATTACC CTATTTTACT GCAGCAGAAA TTATGGGTAT TGGATTTACT
CTACGAGGGT CTGGTAATGG GATAAAATGA CGTCGTCTTT AATACCCATA ACCTAAATGA
```

FIG. 2A

SUBSTITUTE SHEET (RULE 26)

```
      730         740         750         760         770         780
       *           *           *           *           *           *
CAAGAACAAC AAGCAGAAGC AAGATTTGCA CCAGCTAGGA TGCAGTGTAG AGCATGGTAT
GTTCTTGTTG TTCGTCTTCG TTCTAAACGT GGTCGATCCT ACGTCACATC TCGTACCATA

      790         800         810         820         830         840
       *           *           *           *           *           *
CTCGAGGGAC TAGGAAAATT GGGCGCCATA AAAGCTAAGT CTCCTCGAGC TGTGCAGTTA
GAGCTCCCTG ATCCTTTTAA CCCGCGGTAT TTTCGATTCA GAGGAGCTCG ACACGTCAAT

      850         860         870         880         890         900
       *           *           *           *           *           *
AGACAAGGAG CTAAGGAAGA TTATTCATCC TTTATTGACA GATTGTTTGC CCAAATAGAT
TCTGTTCCTC GATTCCTTCT AATAAGTAGG AAATAACTGT CTAACAAACG GGTTTATCTA

      910         920         930         940         950         960
       *           *           *           *           *           *
CAAGAACAAA ATACAGCTGA AGTTAAGTTA TATTTAAAAC AGTCATTAAG  CATGGCTAAT
GTTCTTGTTT TATGTCGACT TCAATTCAAT ATAAATTTTG TCAGTAATTC GTACCGATTA

      970         980         990        1000        1010        1020
       *           *           *           *           *           *
GCTAATGCAG AATGTAAAAA GCCAATGACC CACCTTAAGC CAGAAAGTAC CCTAGAAGAA
CGATTACGTC TTACATTTTT CGGTTACTGG GTGGAATTCG GTCTTTCATG GGATCTTCTT

     1030        1040        1050        1060        1070        1080
       *           *           *           *           *           *
AAGTTGAGAG CTTGTCAAGA AATAGGCTCA CCAGGATATA AAATGCAACT CTTGGCAGAA
TTCAACTCTC GAACAGTTCT TTATCCGAGT GGTCCTATAT TTTACGTTGA GAACCGTCTT
                                                 p25 <------

     1090        1100        1110        1120        1130        1140
       *           *           *           *           *           *
GCTCTTACAA AAGTTCAAGT AGTGCAATCA AAAGGATCAG GACCAGTGTG TTTTAATTGT
CGAGAATGTT TTCAAGTTCA TCACGTTAGT TTTCCTAGTC CTGGTCACAC AAAATTAACA
      -----> p10 Nucleocapsid

     1150        1160        1170        1180        1190        1200
       *           *           *           *           *           *
AAAAAACCAG GACATCTAGC AAGACAATGT AGAGAAGTGA GAAAATGTAA TAAATGTGGA
TTTTTTGGTC CTGTAGATCG TTCTGTTACA TCTCTTCACT CTTTTACATT ATTTACACCT

     1210        1220        1230        1240        1250        1260
       *           *           *           *           *           *
AAACCTGGTC ATGTAGCTGC CAAATGTTGG CAAGGAAATA GAAAGAATTC GGGAAACTGG
TTTGGACCAG TACATCGACG GTTTACAACC GTTCCTTTAT CTTTCTTAAG CCCTTTGACC
                                                -----> POL

     1270        1280        1290        1300        1310        1320
       *           *           *           *           *           *
AAGGCGGGGC GAGCTGCAGC CCCAGTGAAT CAAGTGCAGC AAGCAGTAAT GCCATCTGCA
TTCCGCCCCG CTCGACGTCG GGGTCACTTA GTTCACGTCG TTCGTCATTA CGGTAGACGT

     1330        1340        1350
       *           *           *
CCTCCAATGG AGGAGAAACT ATTGGATTTA TAA
GGAGGTTACC TCCTCTTTGA TAACCTAAAT ATT
                  p10 <-----

          POL ----->
```

FIG. 2B

Sequence Range: 1 to 1353

M G N G Q G R D W K M A I K R C S N A A
-----> p15 Matrix protein

V G V G G K S K K F G E G N F R W A I R

M A N V S T G R E P G D I P E T L D Q L

R L V I C D L Q E R R K K F G S C K E I

D K A I V T L K V F A A V G L L N M T V

S S A A A A E N M F T Q M G L D T R P S

M K E A G G K E E G P P Q A F P I Q T V
p15 <------ ------> p25
Capsid protein

N G V P Q Y V A L D P K M V S I F M E K

A R E G L G G E E V Q L W F T A F S A N

L T P T D M A T L I M A A P G C A A D K

E I L D E S L K Q L T A G Y D R T H P P

D A P R P L P Y F T A A E I M G I G F T

Q E Q Q A E A R F A P A R M Q C R A W Y

L E G L G K L G A I K A K S P R A V Q L

R Q G A K E D Y S S F I D R L F A Q I D

Q E Q N T A E V K L Y L K Q S L S M A N

A N A E C K K P M T H L K P E S T L E E

K L R A C Q E I G S P G Y K M Q L L A E
p25 <------

A L T K V Q V V Q S K G S G P V C F N C
-----> p10 Nucleocapsid

K K P G H L A R Q C R E V R K C N K C G

K P G H V A A K C W Q G N R K N S G N W
K E F G K L
-----> POL (-1 ORF)

K A G R A A A P V N Q V Q Q A V M P S A
E G G A S C S P S E S S A A S S N A I C

P P M E E K L L D L
T S N G G E T I G F I
p10 <-----
POL ----->

FIG. 2C

INTERNATIONAL SEARCH REPORT

Inte. ɔnal Application No
PCT/US 96/08639

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/85 C12N15/49 C12N5/16 C12P21/00 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VIROLOGY, vol. 183, 1991, pages 288-297, XP002013369 S. MORIKAWA ET AL.: "Analyses of the requirements for the synthesis of virus-like particles by FIV gag using baculovirus vectors" *see the whole article* ---	1-18
A	WO,A,93 01278 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 21 January 1993 ---	
A	US,A,5 275 813 (J.K. YAMAMOTO ET AL.) 4 January 1994 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 1996	30. 10. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Marie, A

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 96/08639

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9301278	21-01-93	AU-A-	2299892	11-02-93
		US-A-	5510106	23-04-96
US-A-5275813	04-01-94	US-A-	5037753	06-08-91
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		US-A-	5118602	02-06-92